RHEOLOGY OF REDUCED-FAT MOZZARELLA CHEESE

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INTRODUCTION

Reduction of dietary fat by Americans has led to the introduction of various low-fat dairy products. Consumption of Mozzarella cheese in the U.S. has reached 3.5 kg per person per year (USDA, 1993), making this variety an appropriate target for fat reduction. Our laboratory has developed a "light" Mozzarella containing $<10\%$ fat (Tunick et al., 1991, 1993a, 1993b) which is currently being tested for use in the National School Lunch Program. The effects of cooking temperature, moisture level, and storage time were examined to determine which combination produces the best product. Meltability studies were of particular interest since three-fourths of the Mozzarella cheese produced in the U.S. is used in pizza (Kindstedt, 1993). Homogenization of cheesemilk was also investigated as a possible means of enhancing textural properties and because homogenization is required when recombined milks are used for cheesemaking. In this study, texture profile analysis, small amplitude dynamic oscillatory shear measurements, and meltability measurements were used to compare the rheological properties of low-fat and full-fat Mozzarella cheeses.

MATERIALS AND METHODS

Cheesemaking

Low-fat (LF) and high-fat (HF) Mozzarella cheeses were prepared as described by Malin et al. (1993) and Tunick et al. (1991, 1993a, 1993b). Each type of cheese was prepared from 22.7 kg of milk and one batch was prepared on a given day. Each batch was standardized with cream or skim milk to the desired fat level prior to pasteurization at 63°C for 30 min. LF milk was standardized to 1.0% milk fat and HF milk to 3.5% milk fat. The milk used for homogenized-milk cheeses then underwent two-stage homogenization at 63°C at pressures totaling 10,300 or 17,200 kPa; in each case the second stage was 3450 kPa.

Cheese milk at 32.4°C was inoculated with 125 ml of CR7 starter culture* (Marschall-Rhône Poulenc, Madison, WI), described as 50% *Streptococcus salivarius* ssp. *thermophilus* and 50% *Lactobacillus delbrueckii* ssp. *bulgaricus*. After the pH decreased 0.1 unit, 4.4 g of single strength calf rennet (Chr. Hansen's Laboratory, Milwaukee, WI) was added. Following a hold time of 35 min, the curd was cut and held another 15 min. High-moisture (HM) cheeses were stirred at 32.4°C for 10 min and held at that temperature for 90 min. After the 15-min hold period, low-moisture (LM) curd was heated over a 45-min period to 45.9°C and held at that temperature for 50 min. For both types of cheese, the whey (pH 6.3-6.4) was then drained and the curd was rinsed and cut into slabs. When the pH reached 5.2-5.3, the slabs were covered and iced overnight. The next day, the curd was divided into eight parts and stretched and kneaded multidirectionally by hand for 7 min in 70°-80°C water. The samples were pressed into 224-ml polyethylene cups, cooled, removed from the cups, brined 2 hr in 23% salt solution, blotted dry with clean paper towels, and vacuum sealed in pouches. Refrigerated samples were stored at 4°C for up to 6 wk. Frozen samples were stored at -20°C for 8 wk, thawed at 4°C, and held at that temperature for 3 wk.

Microscopy

Samples for scanning electron microscopy (SEM) were removed from the interior of the cheese at 0 and 6 wk, diced into rectangular blocks approximately 5 x 2 x 2 mm, and immersed in a solution of 1% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.2) at room temperature for 1-2 hr prior to storage at 4°C (Tunick et al., 1993a). Samples were then washed in cacodylate buffer, dehydrated in a graded series of ethanol solutions, extracted with three changes of chloroform, transferred into ethanol, freeze-fractured in liquid nitrogen, thawed into ethanol, and dried at the critical point in carbon dioxide. The dried blocks were mounted on aluminum stubs, coated with a thin layer of gold in a DSM-5 Cold Sputtering Module (Denton Vacuum, Inc., Cherry Hill, NJ) and examined by secondary electron imaging in a JEOL 840A scanning electron microscope (JEOL USA, Peabody, MA).

Textural and Rheological Analyses

Texture profile analysis was performed at 1 and 6 wk as previously described (Tunick et al., 1990), with hardness and springiness being determined at 22°-24°C using an Instron Universal Testing Machine model 4201 (Instron, Inc., Canton, MA). Specimens approximately 14 mm high and 14 mm in diameter were removed from the interior of the cheese sample. The elastic modulus (G′) and viscous modulus (G″) were determined with a Rheometrics Dynamic Analyzer RDA-700 (Rheometrics, Inc., Piscataway, NJ) at 22°-24°C at a frequency of 100 rad/s (Tunick et al., 1990). Three disks, 25.4 mm in diameter and 4-5 mm thick, were removed from the interior of the cheese and glued with cyanoacrylate bonding agent to pairs of parallel plates for the analyses.

Meltability

Meltability was determined at 1 and 6 wk by the Schreiber test (Kosikowski, 1982; Park et al., 1984). Three disks, 5 mm thick and 37 mm in diameter, were removed from the interior of a cheese, placed on glass Petri dishes, and heated in an oven at 232°C for 5 min. The dishes holding the melted disks were cooled on a flat surface and placed on

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a target graph containing numbered concentric circles starting at a diameter of 37 mm (labeled 1) and increasing by 5 mm (labeled 2, 3, 4, etc.). The outer edge of each melted sample was measured in six places and averaged.

Other Analyses

The moisture content of the samples was measured by the forced-draft oven method (AOAC, 1990) and the fat content was determined by the modified Babcock test (Kosikowski, 1982). These analyses were performed at 1 wk only. Percentage of fat in dry matter (FDM) was calculated as follows:

$$\%FDM = \frac{\%fat}{100 - \%moisture}$$

Percentage of moisture in nonfat substance (MNFS) was obtained as follows:

$$\%MNFS = \frac{\%moisture}{100 - \%fat}$$

Linear regressions relating hardness, springiness, G′, G″, and meltability to MNFS were performed; in each case $P < 0.05$.

RESULTS AND DISCUSSION

Microstructure

Secondary electron images of LF, HM Mozzarella at 0 wk (Figure 1a) and 6 wk (Figure 1b) show that the lipids tended to aggregate with time, which was evidently due to proteolytic breakdown of the casein matrix which holds the fat globules in place (Tunick et al., 1993a). This effect was also observed with HF, LM Mozzarella (Figures 1c and 1d). The matrix comprised about two-thirds of the projected surface area in the LF cheese and about one-half of the surface area in the HF cheese. Bacteria remaining from the starter culture usually collected at the surface of the fat droplets, a tendency first noted by Dean et al. (1959). The ratio of chains of *S. thermophilus* to rods of *L. bulgaricus* was approximately 9:1 in all samples; the same ratio has been observed in Mozzarella by Kiely et al. (1992). The LF cheese, which was prepared at a temperature more conducive to bacterial survival, contained about 50% more bacterial colonies than the HF cheese. At 6 wk, the LF cheese contained 25% fewer colonies than at 0 wk, whereas the HF samples displayed a 47% decrease. The release of proteolytic enzymes from these bacteria, as well as the action of plasmin and rennet, apparently contributed to casein proteolysis (Tunick et al., 1993a).

Texture Profile Analysis

The effects of storage time, fat content, and moisture content on Mozzarella cheese hardness can be seen in Figure 2. Proteolysis during refrigerated storage causes partial breakdown of the casein network, which accounts for the reduced hardness of the samples with time (Tunick et al., 1993a, 1993b). Proteolysis also took place while the frozen samples were being thawed. The LF cheeses, which contained 9-11% fat (21-25% FDM),

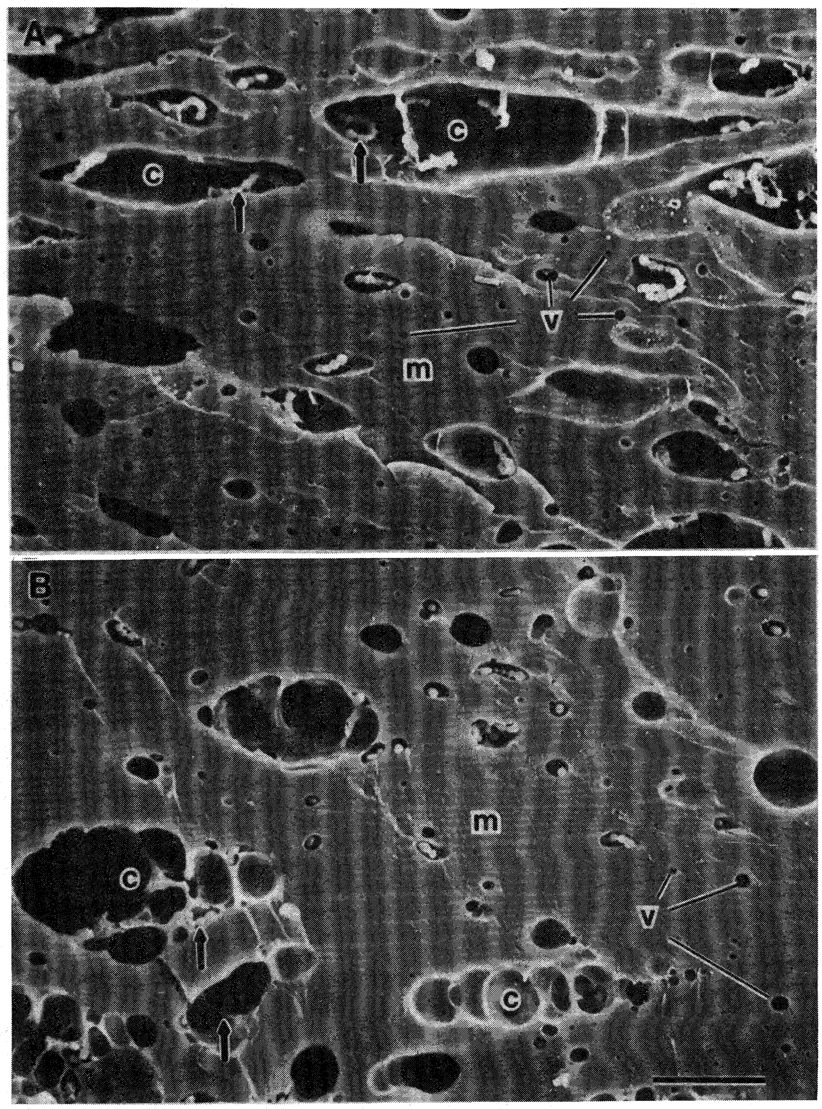

Figure 1. Secondary electron images of (A) LF, HM Mozzarella at 0 wk, (B) LF, HM Mozzarella at 6 wk. Large cavities (c) and small vesicles (v) correspond to fat globules, and are separated by fractured faces of protein matrix (m). Arrows indicate chains of *S. thermophilus*. Bars indicate 10 μm.

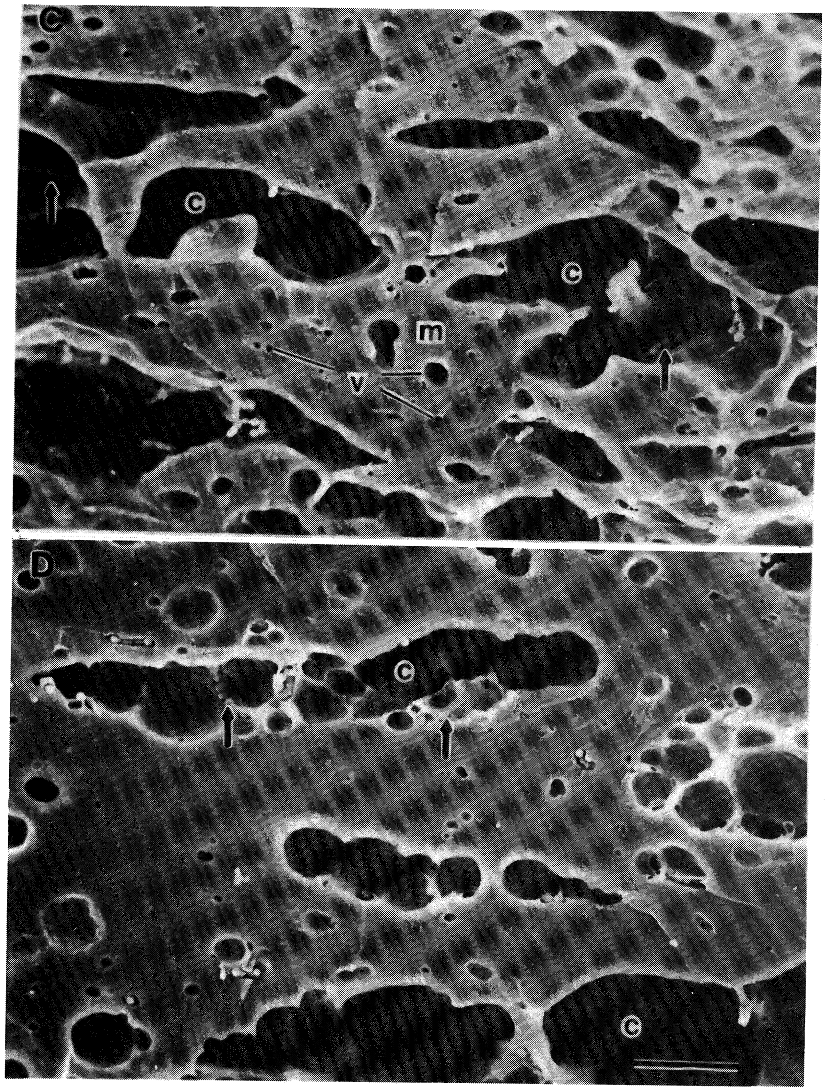

Figure 1 (continued). Secondary electron images of (C) HF, LM Mozzarella at 0 wk, (D) HF, LM Mozzarella at 6 wk. Large cavities (c) and small vesicles (v) correspond to fat globules, and are separated by fractured faces of protein matrix (m). Arrows indicate chains of *S. thermophilus*. Bars indicate 10 μm.

were harder than their HF counterparts, which contained 21-25% fat (43-48% FDM). As seen in Figure 1, more casein has to be deformed per unit volume in LF cheese, resulting in higher hardness values.

LM cheeses, which were cooked at 45.9°C and contained 45-51% moisture, were harder than HM cheeses, which were cooked at 32.4°C and contained 52-58% moisture. Significant trends were observed for MNFS, which is basically a ratio of water to protein

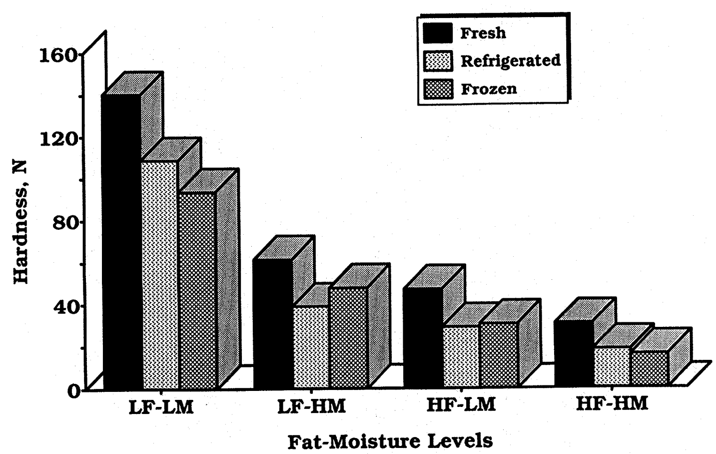

Figure 2. Effects of fat, moisture, and storage on hardness of Mozzarella cheeses made from nonhomogenized milk. LF = low fat, LM = low moisture, HF = high fat, HM = high moisture. Fresh = stored at 4°C for 1 wk, Refrigerated = stored at 4°C for 6 wk, Frozen = stored at -20°C for 8 wk and then at 4°C for 3 wk.

and which affects the texture of cheese (Olson and Johnson, 1990). The linear regression in Figure 3 shows that hardness values decreased as MNFS increased. Water acts as a lubricant in cheese, and a decrease in moisture leads to reduced hydration of protein and less freedom of movement.

The effects of fat, moisture, and storage on springiness can be seen in Figure 4. The springiness values in the fresh LF and fresh HF, LM samples were similar, and decreased during storage because of proteolysis. As with hardness, springiness decreased as MNFS increased (Figure 5).

The texture profile analyses indicate that a reduced-fat Mozzarella can have textural properties similar to those of a full-fat Mozzarella if the MNFS is above 65% and if the cheese is refrigerated for several weeks to allow for proteolysis.

Hardness and springiness were influenced by homogenization pressure. Figure 6 shows the effect of homogenization at 10,300 kPa on the relation between hardness and MNFS. The hardness values are higher than those of the Mozzarella cheeses prepared from nonhomogenized milk (Figure 3), and the decrease in hardness during refrigerated storage is minimal at the lower values of MNFS. The effects are more dramatic when milk homogenized at 17,200 kPa is used (Figure 7). Fat globules are reduced in size during homogenization of milk, resulting in an increase in surface area. The fat globule membrane can cover only part of this increased surface, and casein submicelles cover the

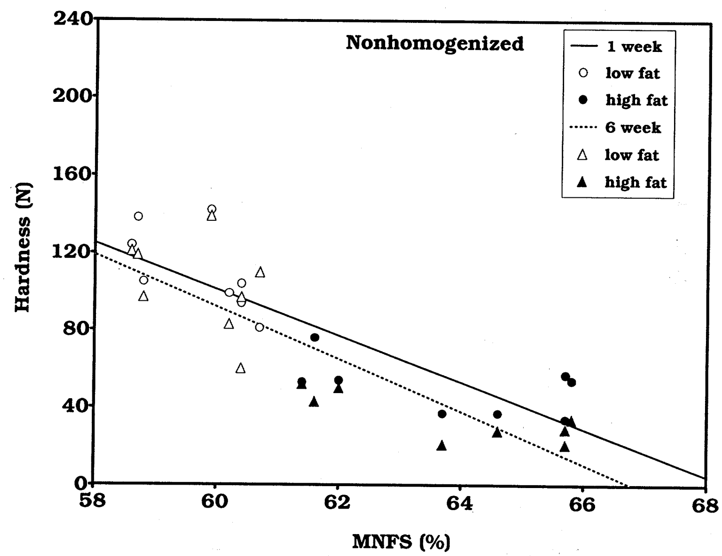

Figure 3. Effect of moisture in nonfat substance on hardness in Mozzarella cheeses made from nonhomogenized milk and stored for 1 wk (R^2 = 0.760) and 6 wk (R^2 = 0.636).

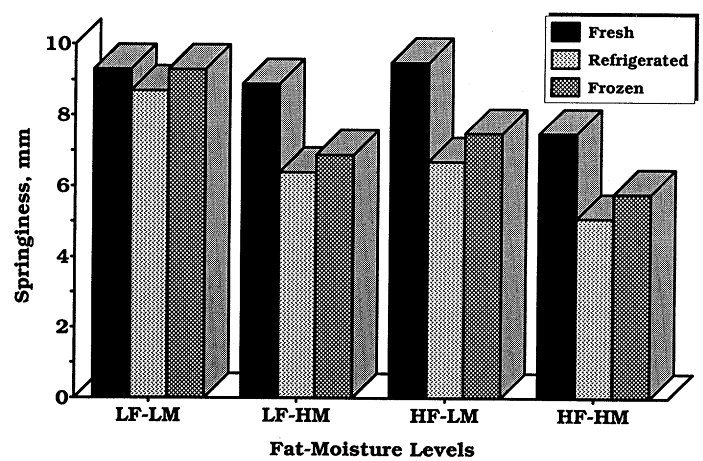

Figure 4. Effects of fat, moisture, and storage on springiness of Mozzarella cheeses made from nonhomogenized milk. LF = low fat, LM = low moisture, HF = high fat, HM = high moisture. Fresh = stored at 4°C for 1 wk, Refrigerated = stored at 4°C for 6 wk, Frozen = stored at -20°C for 8 wk and then at 4°C for 3 wk.

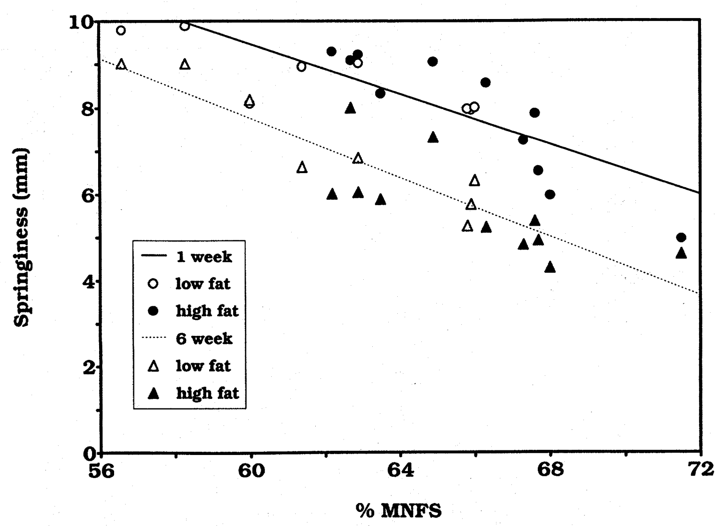

Figure 5. Effect of moisture in nonfat substance on springiness in Mozzarella cheeses made from nonhomogenized milk and stored for 1 wk (R^2 = 0.704) and 6 wk (R^2 = 0.782).

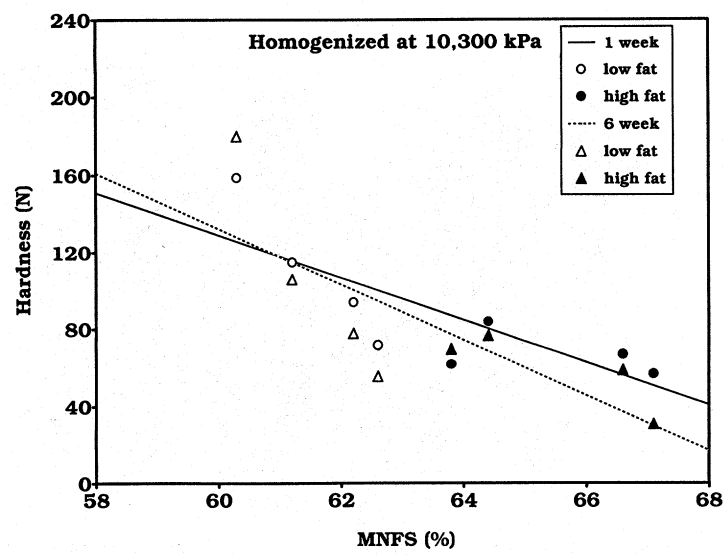

Figure 6. Effect of moisture in nonfat substance on hardness in Mozzarella cheeses made from milk homogenized at 10,300 kPa and stored for 1 wk (R^2 = 0.656) and 6 wk (R^2 = 0.619).

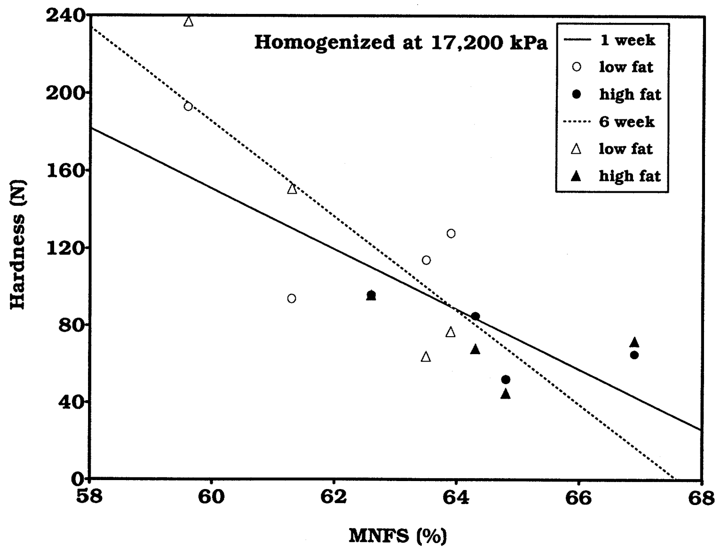

Figure 7. Effect of moisture in nonfat substance on hardness in Mozzarella cheeses made from milk homogenized at 17,200 kPa and stored for 1 wk (R^2 = .548) and 6 wk (R^2 = .759).

rest (Lelievre et al., 1990; McPherson et al., 1984). Interactions between this pseudo membrane and the casein matrix would cause the cheese to exhibit increased hardness and springiness (Tunick et al., 1993b). In addition, conformational changes brought about by homogenization may reduce exposure of cleavage sites in casein, resulting in a reduction in proteolysis.

Elastic and Viscous Moduli

G′ is an indication of the ability to store energy while maintaining structural integrity, and G″ is a measure of the ability to dissipate energy. Both followed the same trends as hardness and springiness -- decreasing with increasing MNFS (Figure 8) and storage time, and increasing with homogenization pressure. G′ was greater than G″ in each specimen, which is expected for viscoelastic solids. The loss tangent, G″/G′, increased with MNFS, since the cheese was becoming more viscous and liquid-like. Increasing the homogenization pressure caused the loss tangent to decrease, as the cheese became more elastic and solid-like.

Meltability

Meltability values increased with fat and moisture content (Figure 9). Proteolysis of the casein results in a decrease in the density of the protein matrix, which allows fat globules to coalesce (Figure 1) and leads to enhanced flow of melted fat. Because proteolysis is enhanced when MNFS is high, Mozzarella meltability increased in higher moisture cheeses (Figure 10).

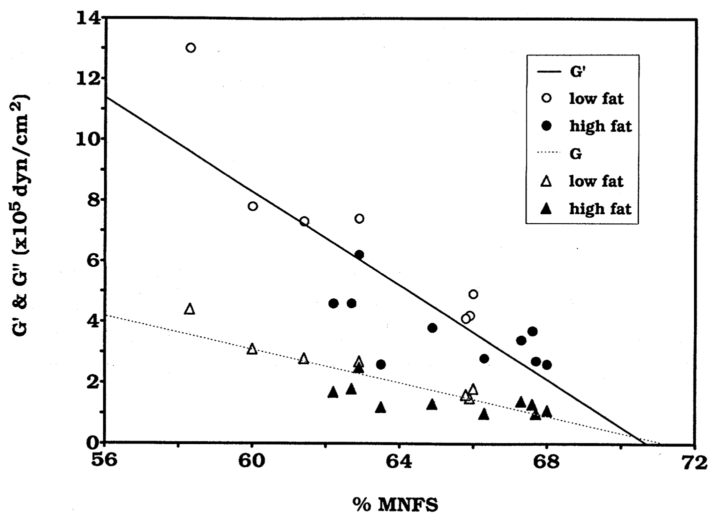

Figure 8. Effect of moisture in nonfat substance on elastic modulus G′ (R^2 = 0.698) and viscous modulus G″ (R^2 = 0.711) in Mozzarella cheeses made from nonhomogenized milk and stored for 1 wk.

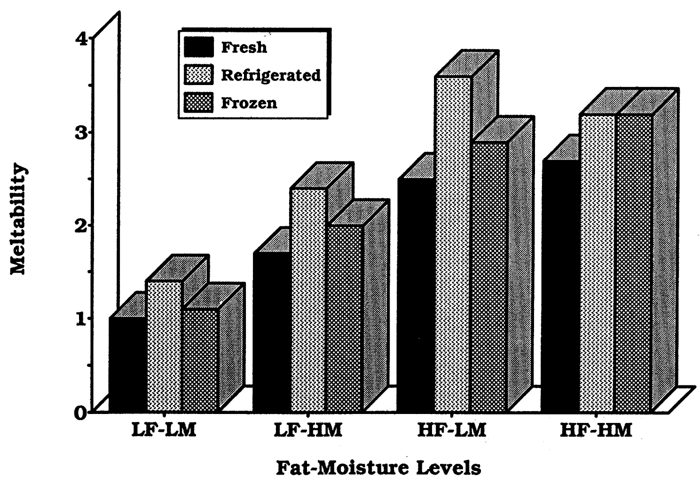

Figure 9. Effects of fat, moisture, and storage on meltability of Mozzarella cheeses made from nonhomogenized milk. LF = low fat, LM = low moisture, HF = high fat, HM = high moisture. Fresh = stored at 4°C for 1 wk, Refrigerated = stored at 4°C for 6 wk, Frozen = stored at -20°C for 8 wk and then at 4°C for 3 wk.

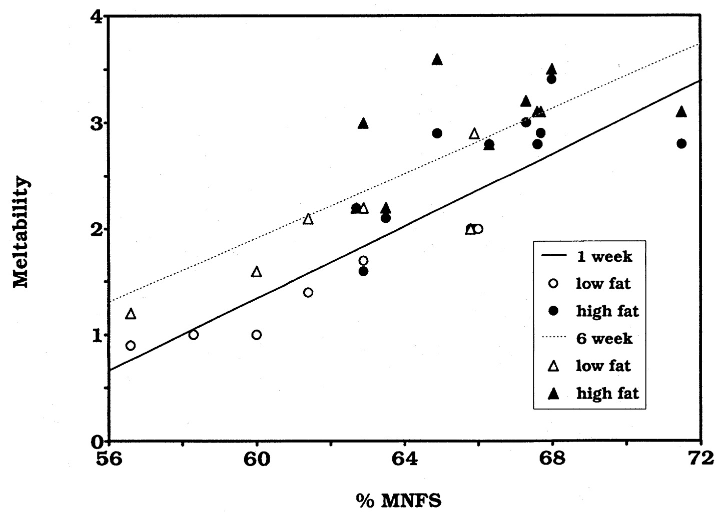

Figure 10. Effect of moisture in nonfat substance on meltability in Mozzarella cheeses made from nonhomogenized milk and stored for 1 wk (R^2 = 0.761) and 6 wk (R^2 = 0.632).

In contrast, meltability decreased as homogenization pressure increased since the pseudo membranes of the smaller fat globules created during homogenization apparently had strong interactions with the casein matrix. Therefore, as noted earlier, the fat globules could not coalesce. All of the cheeses prepared from milk homogenized at 17,200 kPa had relatively low meltability values. However, the LF, HM cheeses made from milk homogenized at 10,300 kPa and refrigerated for 6 wk had an average meltability of 1.7, a value similar to that for cheeses made from nonhomogenized milk. Reduced-fat Mozzarella cheeses ordinarily do not melt well, but the effects of a lower cooking temperature, high moisture, and refrigerated storage combined to result in acceptable meltability.

CONCLUSIONS

Proteolysis during storage causes part of the protein matrix of Mozzarella cheese to break down, leading to decreases in hardness, springiness, elastic and viscous moduli. These effects are also observed when the MNFS level is increased by decreasing the cooking temperature. Meltability is dependent on MNFS and refrigerated storage. A reduced-fat Mozzarella with texture and meltability similar to full-fat Mozzarella can be prepared by controlling moisture level and storage time.

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